International Journal of Pharmacological Research
ISSN: 2277-3312
www.ssjournals.com
Journal DOI:10.7439/ijpr

Effect of two different germplasm of *Mucuna pruriens* seed extracts against some fish pathogens

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Abstract

To investigate the two different germplasm of Mucuna seeds were collected from agro geographical regions was evaluated for its antibacterial activities. Antibacterial activity of the seed extracts was studied against the fish pathogens of *Aeromonas hydrophila, Pseudomonas fluorescens, Vibrio cholera* and *Klebsiella pneumonia* using agar well diffusion method. Results showed that methanol and ethanol extracts showed more potent antibacterial activity than other solvent extracts. The results were expressed as mean ± SD. The results obtained in the study shows that velvet bean black seed extract has more antibacterial activity against fish pathogens. The antibacterial activity of all the Mucuna seed extracts are comparable ad their potential as alternative in the treatment of infectious by these microorganisms was present in the fish. Susceptibility testing is conducted on isolates using drug selected on the basis of their importance to human medicine and use I fish production.

Keywords: Antibacterial activity, Bacterial strains, Velvet bean, Solvent extract

1. Introduction

Fishes are of highly possible of having several bacterial infections, mainly in culture and wild fishes when reared in high density conditions. The outbreaks of diseases cause high economic losses to the fish farmers due to elevated mortality rates and decreased productivity [1] and also bacterial infections of fish and fish products may affect the human health either directly by inducing diseases or indirectly through the settling of antimicrobial residues. Microorganisms which are naturally occurring as pathogens invade the tissue of a host and influence them susceptible to infection [2]. Traditional medicine is a main source of products for developing countries in treating infectious bacteria. India is a hub and it has a rich heritage of knowledge on plant based drugs both for use in preventive and curative medicines. A country like India is very much suited for development of drugs from medicinal plant as it is rich in biodiversity. The primary benefits of using medicines which are derived from plants were relatively safer than synthetic alternative therapeutic benefits and more affordable treatment [3]. Usually these natural products extracted and isolated from plants [4]. These extracts show various medicinal properties especially antimicrobial property. The emergence of multiple drug resistant infectious bacteria, high cost of synthetic compounds as well as undesirable side effects of certain drugs insist on pharmaceutical companies to look for new therapeutic agents from other alternative sources including medicinal plants [5]. Over the past several years, many researchers have been reviewed the antimicrobial activities which had been discovered by intensive efforts.

Mucuna pruriens (*Fabaceae*), velvet bean, is found in Asia, America, Mexico and Eastern Nigeria. In Ayurveda, the decoction of the seeds is known to be used for remedying tuberculosis, cancer and diabetes. It is also used to prepare various formulations, which are used as medicines or alleviating certain diseases [6]. Seeds of this wild legume are widely used for treating male sexual dysfunction in Unani Medicine [7]. Over the past two decades, intensive efforts have been made to discover clinically useful antibacterial/antifungal drugs [8-10]. *Mucuna pruriens* possess a wide range of pharmacologic activities such as antimicrobial activity [11], anti-protozoal activity [12], anti-inflammatory activity[13], neuroprotective activity [14], anti diabetic activity [15], antioxidant activity [16]. The present investigation was undertaken to evaluate antibacterial activity of seed extracts of two different germplasm of *Mucuna pruriens* against fish pathogens viz *Aeromonas hydrophila, Pseudomonas fluorescens, Vibrio cholera* and *Klebsiella.*

2. Materials and methods

2.1. Collection of Seeds

The *Mucuna* seed germplasm (white-colored and black-colored seed coat), were collected from Tamil Nadu, Western Ghats, South India, After drying thoroughly in sunlight for 2-3 days, the parts were thrashed to remove mature seeds; the seeds after thorough cleaning were stored in plastic containers at room temperature (25°C) until further use.

2.2. Preparation of Seed samples

Dry mature seeds of different accessions (10 g each) were powdered in a Wiley Mill to 60-mesh size with suitable precaution to avoid contamination of samples. The powders were stored in plastic containers at room temperature (25°C) until further use.

2.3. Solvent Extraction

Solvent systems used for the extractions were acetone, ethanol, chloroform, petroleum ether, hexane, methanol and water. Soxhlet and flask extraction procedures were adapted for extraction. 10g of each powered samples were packed in muslin cloth and used for extraction by soxhlet apparatus at a temperature below the boiling temperature of each solvent. A portion of the powdered plant samples was soaked in the conical flask containing solvent, wrapped with aluminum foil and placed in shaker for 48 hours at 120-130 rpm. After 48 hours, the extracts were filtered using Whatman filter paper No: 1. the solvent was evaporated and the residue was dissolved in sterile dimethyl sulfoxide (DMSO-9:1) in 50 mg/ml concentration. The extract was filtered using 0.22 micro filters (Type GV- Millipore) and stored at 4°C for further antibacterial activity study.

2.4. Screening for Antibacterial activity

2.4.1. Bacterial strains

The seed extracts were assayed for antibacterial activity against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Vibrio cholera* and *Klebsiella pneumoniae* were obtained from the Department of Biotechnology, SRM University, and Chennai, India. The agar well diffusion method was used to determine the inhibitory effects of the seeds extracts against the isolates [17, 18]. The bacterial isolates were first grown in nutrient broth for 18 h at 37°C, then 0.2 ml of the broth culture of the isolates were aseptically inoculated onto a molten nutrient agar which had been cooled to 45°C, mixed gently and poured into sterile petridishes and allowed to set. The suspension was diluted with sterile distilled water to obtain approximately 10^{-5} CFU/ml. These were delivered into wells (8 mm diameter) bored unto the surface of the inoculated nutrient agar plates. The extracts were allowed to diffuse into the medium for 30 min. The plates were incubated at 37°C for 24 to 48 h. The zones of inhibition were measured in millimeter diameter using meter rule [19].

3. Results

A total of six extracts such as ethanol, Methanol, Chloroform, Acetone, Hexane and Aqueous were examined against the isolated fish pathogens. The anti bacterial activity of *Mucuna pruriens* seed extracts as two forms as black and white colored germplasm against Aeromonas *hydrophila*, *Pseudomonas fluorescens*, *Vibrio cholera* and *Klebsiella pneumonia* were illustrated in (Table 1& Fig-1 and 2).

Table 1: Antibacterial activity of *Mucuna pruriens* black colored and white colored germplasm against fish pathogens.

Name of species	Zone of Inhibition(mm)														
	Ethanol		Methanol		Chloroform		Acetone		Hexane		Aqueous		Solvent control	Positive control	
	BS	WS	BS	WS	BS	WS	BS	WS	BS	WS	BS	WS	-	BS	WS
A. hydrophila	18	17	16	15	15	14	13	13	12	11	10	10	-	20	19
P. fluorescens	16	15	15	14	13	13	12	11	10	9	8	8	-	19	18
V. Cholera	14	13	12	11	11	10	9	9	8	7	7	7	-	19	18
K. Pneumoniae	13	12	11	10	9	8	8	7	7	6	7	7	-	18	17

BS-Mucuna black colored seed; WS-Mucuna white colored seed; Concentration of extract- 100µl/well, (-) - No zone of inhibition observed, Positive control- chloromphenicol (10 µg/ml), Solvent control - 10% DMSO.

Fig- 1: Antibacterial activity of *Mucuna pruriens* black coloured germplasm.

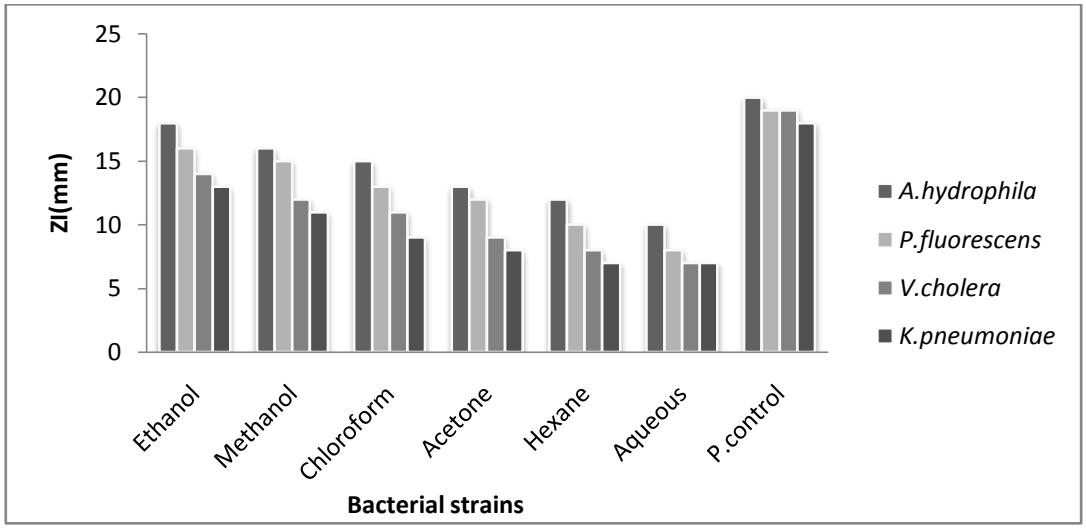
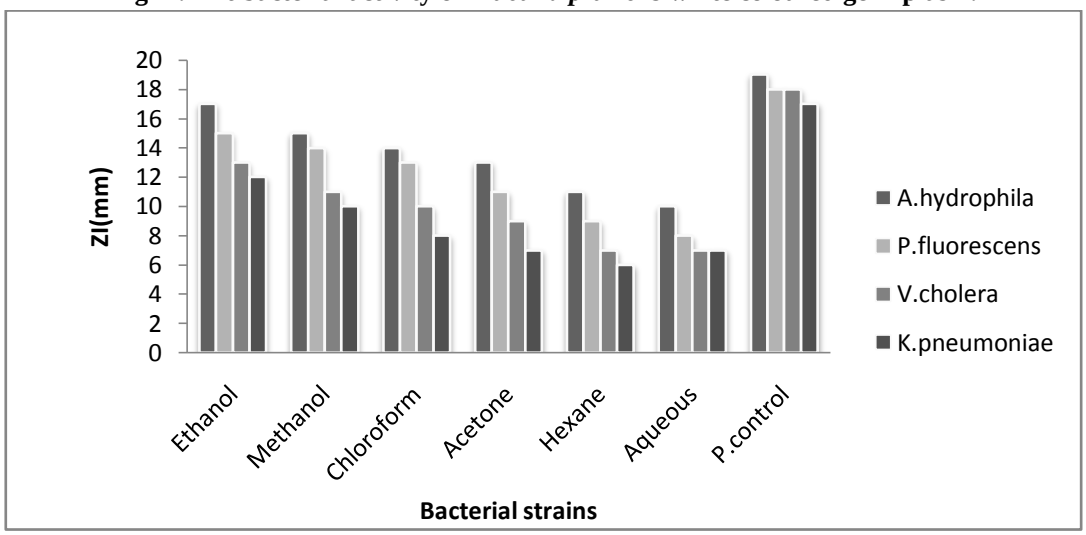


Fig- 2: Antibacterial activity of *Mucuna pruriens* white coloured germplasm.



4. Discussion

Chloromphenicol, standard antibiotic, was used for comparing with the seed extracts .The present study results showed that ethanol extracts of *Mucuna pruriens* (black colored) gave the widest spectrum activities that inhibited the growth of all studied pathogens with the maximum zone of inhibition 18 mm for *A.hydrophila*, 16 mm for *P.fluorescens*, 14 mm for *V. cholera* and 13 mm for *K. Pneumoniae*. Likewise white colored germplasm of *Mucuna pruriens* also showed high variations compare to other extracts such as 17 mm for *A.hydrophila*, 15 mm for *P.fluorescens*, 13 mm for V. *cholera* and 12 mm for *K. Pneumoniae*. The methanol extracts of both black and white colored germplasm demonstrated maximum zone of inhibition against *A.hydrophila* of 16 mm and 15 mm, *P.fluorescens* of 15mm and 14 mm, *V. cholera* of 12 mm and 11 mm and finally 11 mm and 10 mm showed respectively for the *K. Pneumoniae*.

It has been followed by the chloroform extracts of *Mucuna pruriens* black and white colored germplasm against the *A.hydrophila* (15 mm &14 mm), *P.fluorescens* (13 mm &13 mm) *V. cholera* (11 mm &10 mm) and *K. Pneumoniae* (9 mm & 8 mm).The acetone extracts of black colored germplasm of *Mucuna pruriens* against the 13 mm for A.hydrophila, 12 mm for *P.fluorescens*, 9 mm for *V. Cholera* and 8 mm for *K. Pneumoniae*. Likewise, the acetone extracts of white colored germplasm of *Mucuna pruriens* against the 13 mm for *A.hydrophila*, 11 mm for *P.fluorescens*, 9 mm for *V. cholera* and 7 mm for *K. Pneumoniae*. It has been followed by the Hexane extracts of *Mucuna pruriens* black and white colored germplasm against the *A.hydrophila* (12 mm &11 mm), *P.fluorescens* (10 mm & 9 mm) *V. cholera* (8mm&7mm) and *K. Pneumoniae* (7mm&6mm). The Aqueous extracts of both black and white colored germplasm demonstrated zone of inhibition against *A.hydrophila* of 10mm and 10mm, *P.fluorescens* of 8 mm and 8 mm, *V. cholera* of 7mm and 7 mm and finally 7 mm and 7mm showed respectively for the *K. Pneumoniae*. The control for both black and white colored germplasm of *Mucuna pruriens* showed zero percent inhibition against the pathogens viz., *A.hydrophila*, *P.fluorescens*, *V. cholera* and *K. Pneumoniae.*

5. Conclusion

In the present day scenario, increase in antibiotic resistance in a wide range of bacterial species is the major problem around the world. Medicinal plant or herbal drugs are the major resources to counteract this problem. The Mucuna seed extract exhibit certain bio active compounds responsible for the antibacterial activity. The results also show that the methanol and ethanol extracts of seeds significantly varied in their antibacterial potential. The inhibition zones produced by velvet bean seed extract were less than those produced by standard positive control drug. Between two germplasm, black colored germplasm was registered for higher levels of antibacterial activity than white coloured germplasm. Ethanol and methanolic solvents extracts of seed seemed to be brought better antibacterial activity studied in the present report. Antibacterial activity of seed extract is highly comparable with standards. Further in vivo studies and investigations on the isolation and identification of active components in these seeds may lead to chemical entities with potential for clinical use in the prevention and treatment of cataract.

Acknowledgement

We express our sincere gratitude to our Director, Dr. C. Muthamizhchelvan, Engineering and Technology and Dr. M. Vairamani, Dean, School of Bioengineering, SRM University for their continuous support and encouragement towards this study.

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